EARLY DETECTION OF INFLAMMATION USING INFRARED THERMOGRAPHY

1. FIELD OF THE INVENTION

The invention relates to the use of infrared thermography imaging in animals for the early detection of inflammation.

2. BACKGROUND OF THE INVENTION

Inflammation plays a fundamental role in host defenses and the progression of immune-mediated diseases. The inflammatory response is initiated in response to tissue injury (*e.g.*, trauma, ischemia, and foreign particles) and infection by a complex cascade of events, including chemical mediators (*e.g.*, cytokines and prostaglandins) and inflammatory cells (*e.g.*, leukocytes). The inflammatory response is characterized by increased blood flow, increased capillary permeability, and the influx of phagocytic cells. These events result in swelling, redness, warmth (altered heat patterns), and pus formation at the site of injury.

A delicate well-balanced interplay between the humoral and cellular immune elements in the inflammatory response enables the elimination of harmful agents and the initiation of the repair of damaged tissue. When this delicately balanced interplay is disrupted, the inflammatory response may result in considerable damage to normal tissue and may be more harmful than the original insult that initiated the reaction. In these cases of uncontrolled inflammatory responses, clinical intervention is needed to prevent tissue damage and organ dysfunction. Diseases such as Rheumatoid Arthritis, Osteoarthritis, Crohn's disease, psoriasis, or inflammatory bowel disease, are characterized by chronic inflammation.

Early detection and localization of inflammation is a critical step in the implementation of appropriate treatment of a subject. However, non-invasive techniques for the detection of inflammation remain elusive. A variety of techniques including computed tomography (CT), magnetic resonance imaging (MRI), ultrasonography, and scintigraphic imaging are used to attempt to image secondary effects or markers of inflammation. However, CT, MRI, and ultrasonography rely on anatomical changes that result from inflammation, which occur late in the inflammatory response (van der Laken, C.J., et al., 1998, European Journal of Nuclear Medicine 25: 535-546). Therefore, these techniques are not useful for detecting the early phase in the development of inflammation. Scintigraphic imaging is a non-invasive method of scanning the entire body using radiopharmaceuticals (*e.g.*, radiolabeled receptor-specific small proteins and peptides), which specifically bind to receptors abundant in the area of inflammation. The use of

radiopharmaceuticals for imaging inflammation is limiting because it requires: (i) that the radiopharmaceutical specifically interacts with its receptor; (ii) that the radiopharmaceutical have a high affinity for its receptor; (iii) that the radiopharmaceutical specifically localizes to the site of inflammation, which is dependent on the receptor expression in the inflammatory response; (iv) that the receptor is accessible to the radiopharmaceutical; (v) that the radiopharmaceutical have high and early uptake; (vi) that the radiopharmaceutical is rapidly cleared; (vii) that the radiopharmaceutical does not accumulate in non-targeted tissues and result in high background; and (viii) that the radiopharmaceutical is not toxic (van der Laken, C.J., et al., 1998, European Journal of Nuclear Medicine 25: 535-546). The induction of a biological response by a radiopharmaceutical is a major drawback of using scintigraphic imaging. In addition to these technologies, inflammation may also be detected by feeling or visual observance of the site of injury or pain. However, this method is only useful for detecting the late stages in the development of inflammation.

The inability to diagnose and image inflammation *in vivo* continues to be a major obstacle to the successful treatment of inflammatory disorders. Currently, the only viable method for diagnosing inflammatory disorders, such as fibrosis, is by biopsy. This method is invasive and often results in an amount of healthy tissue being removed along with the tissue suspected of being affected by inflammation. Therefore, a great need exists for an accurate, non-invasive, rapid, and inexpensive method for detecting inflammation.

2.1 MASTITIS

Mastitis is an inflammation of the mammary gland normally caused by a bacterial or mycotic pathogen. The disease is of great concern in the dairy industry, where significant economic loss can occur due to the requirement to not use the affected milk for human consumption and due to the shortened milking life of the affected animals. The etiology of the disease is well described in the literature pertaining to this topic, *e.g.*, see, Siegmund et al., 1973, The Merk Veterinary Manual 4th ed., Merck and Comp. Rathway, N.J.; Blood et al., 1983, Veterinary Medicine 6th ed., Bailliere Tindall , London.

The successful treatment of mastitis is possible using a variety of animal management, milking hygiene and antibiotic agents. However, given the expense and labour for the treatment of mastitis, treatment is usually not initiated until the condition is diagnosed clinically.

Numerous mastitis tests have also been proposed, including most recently the use of electrical conductivity of the milk (Notsuki et al., 1983, Proceedings of the World Conference on Animal Production Vol 2., 891-892; Datta et al., 1984, Transactions of the American Society of Agriculture Engineers 27:1204-1210; Batra, T.R. and McAllister, A.J.,

1984, Canadian J. Anim. Sci. 64:305-312; Maatje, K. and Rossing, W., 1991, Mastitis Newsletter 16:6-7; Lake et al., 1991, J. Dairy Sci. 59:11-19; Biagetti, D.R., 1992, Rivista-di-Ingegneria Agraria 23:200-207; Nielsen et al., 1992, J of Dairy Sci. 75: 606-614; Tongel et al., 1994, Proceedings 3rd International Dairy Housing Conference, Orlando, Florida, 257-262). In addition to electrical conductivity, the use of milk components have been suggested as good indicators of mastitis, including such elements as sodium, chloride, potassium, lactose and bovine serum albumin (BSA) (Fernando et al., 1985), milk temperature (Datta et al., 1984, Transactions of the American Society of Agriculture Engineers 27:1204-1210; Rossing et al., 1984, Proceedings of the National Conference American Society of Agricultural Engineers, Chicago, 606-613; Jarman et al., 1986, J. Dairy Sci. 69:(suppl 1.) 178), milk pH (Mijnen et al., 1983, Netherlands Milk and Dairy Journal 37:65-77), milk anti-trypsin (Mattila et al., 1985, J. Dairy Sci. 68:114-122) as well as general milking information such as volume or yield (Nielsen et al., 1994, Veterinary Research 25:285-289). Numerous patents have been issued describing the methods of mastitis detection, particularly for the use of electrodes or a variety of electrical conductivity tests for milk (U.S. Patent No. 3,989,009; U.S. Patent No. 3,968,774; U.S. Patent No. 4,156,179; Australian Patent Application AU A178 553/81; U.S. Patent No. 5,302,903; U.S. Patent No. 5,416,417).

All of these aforementioned procedures can be useful. However, none are particularly effective at early detection (*e.g.*, within the first few hours) of mastitis onset and, as described by Batra and McAllister (1984), these aforementioned procedures often have an unacceptably high percentage of false negatives (*i.e.*, failure to identify an infected cow). For example, electrical conductivity is reported to have a 29.4% false negative value and is also shown to be unreliable unless selective milk samples are used (Noksuki et al., 1983, Proceedings of the World Conference on Animal Production Vol 2., 891-892).

Mastitis is currently detected predominantly by the use of inflammatory tests such as the "Wisconsin Mastitis Test" or CMT, which as described by Siegmund (1973, page 817) is a rather time consuming laboratory type diagnostic method which will indicate the relative leukocyte or somatic cell count in the milk of cows suspected of having mastitis. Unfortunately, these types of tests are not particularly effective in detecting the earliest onset or subclinical cases of mastitis. Furthermore, the need to capture the animal and collect milk samples complicates the use of this method. These factors are important in that the earlier the mastitis condition can be detected, the earlier treatments can begin and the higher the likelihood of successful treatment in a shorter period of time.

As mentioned previously, these tests have in common the requirement of collecting and analyzing milk samples from animals suspected of having mastitis. Clinical diagnosis

of the infected animal is also routinely conducted. However, clinical signs of mastitis usually do not occur until the animal has progressed well into the disease state. Furthermore, some diagnostic tools, such as rectal temperature, while usually efficacious, are often not as sensitive as would be desired or are simply impractical. Again, it should be noted that the earlier a diagnosis can be performed, the earlier treatment can be initiated, which results in a lower treatment cost and a more successful outcome. Therefore, there remains a need for an accurate, inexpensive, non-invasive, rapid method for predicting early mastitis onset in dairy animals.

2.2 INFRARED THERMOGRAPHY

Infrared thermography is a non-invasive technique that enables temperatures to be monitored and recorded. Unsuccessful attempts have been made to use infrared thermography in human medicine as a diagnostic aid for a variety of conditions, such as tumor detection and cardiovascular disease (Clark, J.A. and Cena, K., 1972, J. of Mammalogy 54:1003-1007). Infrared thermography has been attempted in veterinary medicine to detect and diagnosis a variety of conditions, such as podotrochlosis in horses (Turner,T.A., 1983, Am. J. Vet. Res. 44:535-539) and clinical damage in an udder (Tsykalo, A.L. et al., 1982, USSR (7):49-50) .

The early infrared thermography detection systems were bulky, complex, and required frequent recharging with liquid nitrogen. Furthermore, the spatial resolution was poor, the exposure time was long, and the minimum resolvable temperature difference was large for the infrared thermography systems. Reliable detection of inflammation was not achieved. In addition, many physicians and veterinarians were not adequately trained to interpret the data from the infrared imagery and there was a high false positive rate. Thus, the infrared thermography was branded as a failure and has not been explored much by the medical or veterinary communities for the past three decades.

3. SUMMARY OF THE INVENTION

The present invention provides a method using infrared thermography for the detection of inflammation in animals. The invention also provides a method using infrared thermography for the diagnosis of infections, diseases or disorders that induce inflammation. The present invention is based on the surprising discovery that temperature differences less than 1 °C are clinically significant. This discovery was made possible by employing an induction model of mastitis, which allowed the Applicants to evaluate inflammation resulting from a known etiology and to compare the infrared characteristics obtained using an infrared camera with outcomes obtained with other diagnostic procedures.

Accordingly, Applicants' discovered that temperature differences less than 1 °C indicate early or subclinical inflammation, and that temperature differences greater than 1 °C indicate later stages of development of inflammation.

4. DESCRIPTION OF THE FIGURES

Figure 1 is a sketch illustrating the main components of the illustrative apparatus of the present invention.

Figure 2 is a side view depicting an illustrative embodiment of the invention.

Figure 3 is a block diagram depicting the electronics found in the imaging system of the present invention.

Figure 4 is a block diagram depicting the electronics found in the flip-out display.

Figure 5 is an illustration of the front of the display panel.

Figure 6 is a graph of rectal temperature and udder infrared thermography values for milking dairy cows having mastitis induced in the left distal quadrant (n=20). Data for both the left and right distal quarters of the udder are shown.

Figure 7 is a graph of Nagase (N-acetyl-beta-D-glucosaminidase) and udder infrared thermography values for milking dairy cows having mastitis induced in the left distal quadrant (n=20). Data for both the left and right distal quarters of the udder are shown.

Figure 8 is a graph of BSA (Bovine Serum Albumin) and udder infrared thermography values for milking dairy cows having mastitis induced in the left distal quadrant (n=20). Data for both the left and right distal quarters of the udder are shown.

Figure 9 is a graph of somatic cell count and udder infrared thermography values for milking dairy cows having mastitis induced in the left distal quadrant (n=20). Data for both the left and right distal quarters of the udder are shown.

Figure 10 is a graph of image area (pixels) for the left and right distal quarters of the udder in milking dairy cows having mastitis induced in the left distal quadrant (n=20).

Figure 11 is a graph is of rectal temperature and udder infrared thermography values for a milking dairy cow (n=1) having mastitis induced in the left distal quarter. Data for both the left and right distal quarters of the udder are shown.

Figure 12 is a graph of NAGase and udder infrared thermography values for the animal of Figure 11. Data for both the left and right distal quarters of the udder are shown.

Figure 13 is a graph of BSA and udder infrared thermography values for the animal of Figures 11 and 12. Data for both the left and right distal quarters of the udder are shown.

Figure 14 is a graph of total temperature values (mean udder temperature x image area) for milking dairy cows having mastitis induced in the left distal quadrant (n=20). Data for both the left and right distal quarters of the udder are shown.

5. DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to the use of infrared thermography for the early or subclinical detection of inflammation in animals. The present invention further relates to the use of infrared thermography in the diagnosis of infections, diseases or disorders that induce inflammation. The present invention provides methods for detecting inflammation of an anatomical structure of an animal, preferably all mammals, more preferably non-human animals. The term "anatomical structure" used herein refers to a body structure of an animal, preferably a tissue or a joint of an animal, which may or may not be symmetrical. In one embodiment, the invention provides for methods of detecting inflammation of all anatomical structures of animals, except the joints. In another embodiment, the present invention provides for methods of detecting inflammation of the joints of all mammals, except humans. In yet another embodiment, the invention provides for methods of detecting inflammation in all non-human mammals, including but not limited to pigs, horses, cows (*e.g.*, *Bos taurus* and *Bos indicus*), dogs and cats.

The invention provides a method for detecting inflammation of an anatomical structure of an animal, comprising the following steps: (i) obtaining an infrared thermographic image of an anatomical structure of an animal; (ii) determining the total temperature of the infrared thermographic image; and (iii) detecting early or subclinical inflammation of an anatomical structure of an animal if there is a change in the mean temperature of less than 1 °C of an anatomical structure relative to the mean temperature of the same anatomical structure of the same animal or a population of animals of the same species obtained from infrared thermographic images taken when there was no inflammation of the anatomical structure. The term "subclinical" as used herein refers to inflammation of an anatomical structure of an animal that has not manifested itself clinically.

The invention also provides a method for detecting inflammation of an anatomical structure of an animal, comprising the following steps: (i) obtaining an infrared thermographic image of an anatomical structure of an animal; (ii) determining the total temperature of the infrared thermographic image; and (iii) detecting late stage development of inflammation of an anatomical structure of an animal if there is a change in the mean temperature of greater than 1 °C of an anatomical structure relative to the mean temperature of the same anatomical structure of the same animal or a population of animals of the same species obtained from infrared thermographic images taken when there was no inflammation of the anatomical structure.

The invention also provides a method for detecting inflammation of an anatomical structure of an animal, comprising the following steps: (i) obtaining an infrared

thermographic image of an anatomical structure of an animal after an event; (ii) comparing the infrared thermographic image obtained to infrared thermographic images of the same anatomical structure of the same animal or a population of animals of the same species prior to the event; and (iii) detecting inflammation of the anatomical structure of the animal if there is a relative difference in the temperature of the anatomical structure of the animal. The term "event" as used herein refers to any activity that may result in inflammation of an anatomical structure of an animal, including surgery.

The present invention provides a method for detecting inflammation of an anatomical structure of an animal, comprising the following steps: (i) obtaining an infrared thermographic image of an anatomical structure of an animal; (ii) obtaining an infrared thermographic image of the symmetrical anatomical structure of the animal; (iii) determining the total temperature of the infrared thermographic images for the symmetrical anatomical structures; and (iv) detecting inflammation of an anatomical structure if the total temperature of the symmetrical anatomical structures differ by greater than a predetermined amount. The term "symmetrical anatomical structure" as used herein refers to an anatomical structure that has symmetry to another anatomical structure of an animal (*e.g.*, one leg compared to another leg of an animal).

The invention also provides a method for detecting inflammation of an anatomical structure of an animal, comprising the following steps: (i) obtaining an infrared thermographic image of the anatomical structure of an animal; (ii) obtaining an infrared thermographic image of the symmetrical anatomical structure of the animal; (iii) comparing the infrared thermographic image obtained to an infrared thermographic image of the symmetrical anatomical structure of the animal; and (iv) detecting inflammation of the anatomical structure of the animal if there is a relative difference in the temperature between the anatomical structure and the symmetrical anatomical structure of the animal.

The present invention further provides a method for detecting when a clinical treatment for treating inflammation of an anatomical structure of an animal was successful, comprising the following steps: (i) obtaining an infrared thermographic image of the anatomical structure of the animal; (ii) determining the total temperature of the infrared thermographic image; and (iii) detecting the successful treatment of inflammation of the anatomical structure by comparing the total temperature of the anatomical structure with the total temperature of the same anatomical structure obtained from the same animal or a population of animals of the species when healthy.

5.1 INDUCTION MODEL OF MASTITIS

The present invention is based upon the surprising discovery that temperature differences less than 1 °C are clinically significant. This discovery was made possible by employing an induction model of mastitis, which displays a known etiology, such that infrared thermal expression could be compared to known outcomes. The use of the induction model has many advantages including: (i) the inflammatory agent is known both in quantitative and qualitative terms; (ii) the exact time of the onset of inflammation is known; and (iii) the exact stage or progression of the inflammation is known. Furthermore, due to the unique anatomy of the udder of a cow, the progression of an infected quarter can be compared to a non-infected quarter. The udder of a dairy cow is unique in that all four quarters are essentially independent in terms of their vascular supply (Sisson, S., The Anatomy of the Domestic Animal. W.B. Saunders Comp., Philadelphia. 4th ed. Revised by J.D. Grossman, page 618), such that inflammation induced in one quarter of the udder through the use of a mastitis induction model does not affect any other quarter of the udder. Hence, the animal can act as its own control.

Briefly, in achieving the invention, one quarter of the udder of a test population of lactating dairy cattle was infected with *Escherichia coli* (*E. coli*) endotoxin and the time course of the resulting inflammation was followed for several days using a variety of analytical tools, including infrared thermography. Over a 72 hour time course, milk samples were obtained from the left (induced) and right (non-induced) distal (hind) quarters of the udder and analyzed for objective indicators of inflammation by conventional analytical procedures. Contemporaneously with the milk samples, infrared thermographic images of the cows were obtained, so that the infrared thermal expression of the animal could be monitored over the course of the induced inflammation.

It was found that within hours after induction of inflammation, significant changes in the thermal expression of the cows could be detected with infrared thermography. This was surprising, in that, as discussed previously, conventional thought would dictate that any temperature changes occurring in subclinical cases of mastitis would be too subtle to detect. Moreover, these changes in thermal expression were observed in all cows in which inflammation was induced, indicating that altered thermal expression, as detected by infrared thermography, is a reliable indicator of inflammation. Significant changes in infrared thermal expression included: (i) a temperature increase; (ii) a more rapid rate of temperature change; and (iii) swelling of the affected quarter of the udder, resulting in a reduction in the symmetry of the thermal expression between the udder quarters with the affected quarter being both hotter and larger. In the present invention, one or more of these changes, detected by infrared thermography, is used to diagnose inflammation.

In one embodiment of the present invention, mastitis in a mammal is detected by: (i) obtaining an infrared thermographic image of a mammary gland of said mammal, said infrared thermographic image providing temperature information about said mammary gland; and, (ii) identifying said mammal as having a high probability of having mastitis if a measure of said temperature information is greater than a predetermined value by at least a predetermined amount. In another embodiment of the present invention, mastitis in a mammal having an udder is detected by: (i) obtaining an infrared thermographic image of one quarter of the udder of said mammal at time 0, said infrared thermographic image providing temperature information about said udder quarter of said mammal; (ii) obtaining an infrared thermographic image of the same quarter of the udder of said mammal at a later time, said infrared thermographic image providing temperature information about said udder of said mammal; (iii) determining a total temperature for a first image, said first image corresponding to said quarter of the udder of said mammal at time 0; (iv) determining a total temperature for a second image, said second image corresponding to said quarter of the udder of said mammal at a later time; and (v) identifying said mammal as having a high probability of having mastitis if the total temperature for said first image differs from the total temperature for said second image by greater than a predetermined amount. In yet another embodiment of the present invention, mastitis in a mammal having an udder is detected by: (i) obtaining images of the two frontal quarters or two rear quarters of the udder of said mammal; (ii) determining the total temperature of a first image, said first image corresponding to one frontal quarter or one rear quarter of the udder of said mammal; (iii) determining the total temperature of a second image, said second image corresponding to the other frontal quarter or the other rear quarter of the udder of said mammal; and (iv) identifying said mammal as having a high probability of having mastitis if the total temperature of said first image differs from the total temperature of said second image by greater than a predetermined amount.

5.2 INFRARED THERMOGRAPHIC CAMERA

Capturing reliable infrared data from live animals is a technical and operational challenge. Moving conscious animals to designated analytical or assessment rooms where equipment, monitors and data collection are fixed permanently, is not always possible. In fact, handling and management procedures by themselves can be stressful to animals resulting in non-steady state or aberrant temperature profiles. Hence, the animal technician or camera operator is often required to move to the animal's environment. Therefore, utilizing a user friendly infrared camera that is installed in the animal's environment or that is portable is an advantage when capturing infrared data from live animals.

In one embodiment of the invention, the infrared thermographic camera is held and operated with one hand, which is a significant advantage when obtaining infrared thermographic images of an anatomical structure of animals. In a preferred embodiment, the portable, hand held camera is light enough to be managed easily. In another embodiment, the infrared thermographic camera is installed in the animal's environment (*e.g.*, a barn). In another preferred embodiment of the invention, the infrared thermographic camera: (i) is designed to operate and function optimally within the range of temperatures normally anticipated in animals displaying inflammation (25°C to 35°C) without recalibration; (ii) is capable of resolving temperature differences of less than 1°C; (iii) has a lens focal length that is optimal for use in closer ranges with animals (*e.g.*, focal length = 7 inches to infinity); (iv) has a wavelength range of 8 to 14 μm; (v) is encased in a hardened, water resistant case, which is compatible for the capture of data in animal environments; (vi) has a flip out display for accurate viewing of the image; and (vii) is capable of compact data storage in the instrument and/or linkage to peripheral monitors. In the examples described, the Inframetrics 760 broadband camera (Inframetrics Co. North Billercia, MA) was used to obtain the infrared thermographic images.

5.3 PROTOCOL FOR INFRARED THERMOGRAPHIC IMAGING

For predicting the early onset of inflammation, each animal or animals suspected of presenting inflammation in a population are scanned from about 1-3 meters away. For detection of inflammation due to mastitis, the preferred range is 175 cm. Infrared thermographic images of all non-human animals are collected preferentially from the distal (hind) view showing a clear display of the back two quarters. However, other images such as the ventral or lateral view would also have utility.

Environmental factors such as motion, extraneous radiant energy, and ambient temperature must be controlled when using infrared thermography to detect inflammation. Motion, for example, can be controlled by immobilizing the animal (*e.g.*, a cow can be tied with a neck chain). Infrared thermographic images should be obtained under cover, shielded from the sun. Preferably, the ambient temperature of the environment should be in the range of 20°C, and most preferably the ambient temperature of the environment should be less than 30°C. Artifacts such as debris on the surface of the animal, scar tissue, irregular patterns of hair length, liniment and wraps should be eliminated to avoid interference with the infrared thermographic image(s). The animal also should be acclimated to the site of the examination for at least ten minutes prior to the examination.

5.4 INTERPRETATION OF INFRARED THERMOGRAPHIC IMAGES

The thermal expression of an animal is determined by obtaining infrared thermographic images. As used herein, the term "infrared thermographic image" is meant to include a scan output in the form of either or both a visual image and corresponding temperature data. The output from infrared cameras used for infrared thermography typically provides an image comprising a plurality of pixel data points, each pixel providing a temperature data point that can be further processed by computer software to generate, for example, mean temperature for the image, or for a discrete area of the image, by averaging the data points over the number of pixels.

It will be appreciated by those of skill in the art that an infrared thermographic image, comprising a plurality of pixels, provides a large number of temperature data points. Therefore, before comparing the temperature information to a predetermined value, determining a rate of temperature change, or determining a difference in total temperature, it is useful to obtain some measure that is representative of the entirety of the temperature information provided by an infrared thermographic image or a part thereof. Selected measures for the temperature information derived from each infrared thermographic image for the subject animal are determined by statistical techniques known in the art. Preferred measures include measures of central tendency, measures of dispersion, and measures of total temperature.

The term "measure of central tendency" as used herein is a statistical measure of a point near the center of a group of data points; without limitation, the term includes the mean, median, and mode. The term "measure of dispersion" as used herein is meant to include statistical measures of spread from the measure of central tendency for the group, and include, without limitation, variance, standard deviation and coefficient of variation. Definitions of these statistical terms may be found in standard statistics texts, such as Steel and Torrie (1980), which definitions are incorporated herein by reference. As used herein, the term "total temperature" means a measure of the central tendency for the temperature information from an infrared thermographic image x image area or image volume expressed in pixels (*e.g.*, if the mean temperature = 20°C and the image is equal to 200 pixels, then the total temperature = 20°C x 200 pixels = 4000 pixels).

An uncalibrated, digitized thermographic image may consist of, for example, 135 X 256 pixels. In analyzing the thermographic image, the relative radiant surface temperature represented by each pixel of the uncalibrated image may be represented by assigning each pixel a numerical value in the range from, for instance, 0 to 255. The pixel values are mapped to actual Celsius temperature by relating them to the maximum and minimum temperature settings of the infrared camera through the following formula:

$$\text{Actual Temperature} = \frac{(\text{max temp setting} - \text{min temp setting}) \times \text{pixel value}}{256}$$

To assist a human operator in viewing the infrared thermographic images on a computer monitor, pseudo colours can be generated by assigning a specific colour to all pixels with temperature values within a certain range.

The entire thermographic image may be processed. In a preferred embodiment, only data for a part of the image corresponding to the area of interest of the animal is analyzed. Known computer analysis procedures, such as planometry, can be used to restrict the image analysis to the selected area of interest of the animal. For each infrared thermographic image obtained for an animal, the image area and the selected image temperature statistics are calculated. Selected statistical measures of the temperature information (each pixel in the infrared thermographic image providing a temperature data point), such as the mean, median, mode, standard deviation, variance, and coefficient of variation can be determined by well-known statistical techniques such as those described by Steel and Torrie (1980). Suitable software for analyzing the thermographic images include Thermogram™ image software (Inframetrics, Inc,. North Billercia, MA) and Viewscan™ Software (Viewscan Ltd., Concord, ON.). Mathematical models using such analytical approaches as neural nets can also utilized to analyze the thermographic image.

In one embodiment of the present invention, temperature differences between symmetrical anatomical structures are compared to detect inflammation. For example, the lack of symmetry between affected and non-affected quarters of an cow's udder can be used to detect mastitis. In a preferred embodiment, the area or volume information is combined with the infrared thermographic temperature to better discern the lack of symmetry between the affected and the non-affected anatomical structure. The area or volume represented by selected portions of the infrared thermographic images can be determined by known techniques.

In an embodiment of the present invention, inflammation of an anatomical structure of an animal is detected if a measure of temperature information for an infrared thermographic image of an anatomical structure of the animal differs by at least a predetermined amount from a predetermined value. The predetermined value may also represent published conventional temperature data representing animals of the same species as the subject animal, which can be adjusted to reflect infrared thermographic temperature values. Alternatively, the predetermined value may be an arbitrary value, the value having been determined through trial and error to be useful for detecting inflammation of an anatomical structure of an animal. Preferably, the predetermined value represents an equivalent measure of temperature information for infrared thermographic images of the particular anatomical structure obtained for members of a population of the same species of

animal being examined when there was no inflammation of the anatomical structure. More preferably, the predetermined value represents an equivalent measure of temperature information for one or more infrared thermographic images of the animal obtained at a time when there was no inflammation of the anatomical structure of the animal, and more preferably, when the animal was healthy.

In a preferred embodiment, a change in the mean temperature of less than 1 °C of an anatomical structure relative to the mean temperature of the same anatomical structure of the same animal or a population of animals of the same species obtained from infrared thermographic images taken when there was no inflammation of the anatomical structure indicates early or subclinical inflammation. In another preferred embodiment, a change in the mean temperature of greater than 1 °C of an anatomical structure relative to the mean temperature of the same anatomical structure of the same animal or a population of animals of the same species obtained from infrared thermographic images indicates late stage development of inflammation. In another preferred embodiment, inflammation of an anatomical structure of an animal is detected if the mean of the temperature information obtained from the infrared thermographic image is preferably greater than 0.2 °C, more preferably greater than 0.1 °C the mean of the temperature information for previously obtained infrared thermographic images of the same animal when there was no inflammation of the anatomical structure. In yet another preferred embodiment, inflammation of an anatomical structure of an animal is detected if the mean of the temperature information obtained from the infrared thermographic image is preferably greater than 0.2 °C, more preferably greater than 0.1 °C the mean temperature obtained from infrared thermographic images for the same anatomical structure of the same species animal when there was no inflammation of the anatomical structure.

In another embodiment of the present invention, inflammation of an anatomical structure is detected if a measure of temperature information for an infrared thermographic image of an anatomical structure of the animal is equivalent to or greater than the predetermined value for the anatomical structure of the animal. Preferably, the predetermined value represents the mean temperature obtained from infrared thermographic images of the same anatomical structure in members of the same species of animal when there is inflammation.

In another embodiment of the present invention, inflammation of an anatomical structure of an animal is detected if the change in temperature obtained by successive infrared images of the same anatomical structure of the same animal is greater than a predetermined rate, preferably greater than a rate of 0.1 °C/hour. Preferably, successive

infrared images of an anatomical structure of an animal are taken every 10, 30 or 60 minutes.

In a further embodiment of the present invention, inflammation of an anatomical structure of an animal is detected if the total temperature of a section of an infrared thermographic image corresponding to one anatomical structure of the animal differs by more than a predetermined amount, preferably 10%, from the total temperature of a section of the infrared thermographic image corresponding to the symmetrical anatomical structure of the animal. The total temperature preferably represents the area or volume of the relevant image section, which can be represented as a number of pixels, multiplied by the mean pixel temperature.

In an embodiment of the present invention, area or volume information alone, independent from temperature information, can be used to detect inflammation of an anatomical structure of an animal. Inflammation of an anatomical structure of an animal is detected if the area or volume of a section of an infrared thermographic image corresponding to one anatomical structure of the animal differs by more than a predetermined amount, preferably 10%, from the area or volume of a section of the infrared thermographic image corresponding to the symmetrical anatomical structure of the animal.

The infrared thermographic temperature information can be normalized or standardized by compensating the temperature information to account for one or more of the following: (i) the state of lactation of the animal; (ii) the state of parity of the animal; (iii) the circadian temperature variation; (iv) the diurnal temperature variation; (v) the animal breed; (vi) the animal housing conditions; or (vii) the geographic location. An adjustment for the state of lactation of an animal would be useful for normalization because animals in early lactation typically have a higher milk production and hence larger udders. An adjustment for the state of parity of an animal would also be useful for normalization because cows, for example, typically in their third or fourth parity will have larger udders than cows in their first parity. Adjustments to normalize the infrared thermographic data depending on when an animal is observed during the day should be performed because an animal's normal temperature will fluctuate over a 24 hour period. The temperature change during the day will also vary with the time of day a cow is milked, hence, a normalization scale would be useful. Adjustments to normalize infrared thermographic data obtained from different breeds of animals should be performed because of differences in their anatomical structures. Furthermore, adjustments to normalize the infrared thermographic data obtained from animals housed differently (*e.g.*, in barns with concrete floors versus in barns with rubber matts) and in different geographic locations (*e.g.*, Edmonton versus Orlando) should be performed.

6. EXAMPLE

In order that the invention described herein may be more fully understood, the following example is set forth. It should be understood that this example is for illustrative purposes only and is not to be construed as limiting this invention in any manner.

MATERIAL & METHODS

Twenty mature lactating Holstein cows at 120 days post-partum were housed at the Agriculture and Agri-Food Canada Dairy Research Unit at Lennoxville, Quebec, and were managed in a manner consistent with and representative of the dairy industry in North America, and in compliance with the Canadian Council of Animal Care Guidelines. The left distal quarter of the udder of each animal was infused with 10 μg of *E. coli* endotoxin (serotype 055:B5, Sigma-Aldrich Co.) in 10 ml of sterile saline.

Fifteen of the cows were additionally treated with experimental inflammation inhibitors. The twenty cows were divided into four treatment groups of five animals each as follows: (i) control, no prophylactic treatment; (ii) aminoguanidine introduced into the cistern of the infected teat; (iii) arginine methyl ester introduced into the cistern of the infected teat; and (iv) dexamethasone introduced into the cistern of the infected teat. The treatments were applied in an effort to attenuate the mastitis response.

Milk samples from the control (right distal) and induced (left distal) quarters of each animal were collected at 13 hours and 1 hour pre-induction and also at 2, 6, 9, 12, 24, 36, 48, 60 and 72 hours post-induction. The milk samples were analyzed for objective indicators of mastitis by conventional analytical procedures as discussed hereinafter. Infrared thermographic images of both distal quarters were simultaneously taken at these times and at 0.5, 1, 1.5, 2 and 2.5 hours post-induction. An Inframetrics 760™ broadband camera (Inframetrics Inc., North Billerica, MA) fitted with a 0.5 X lens was used to collect the infrared images. Working indoors, images of the posterior surface of the udder of each animal were obtained from a distance of 2.1m. The Images were recorded on videotape with a videocassette recorder. The analog Images were captured and digitized using a computer equipped with a Matrox Meteor™ video card (Matrox Electronic Systems Ltd., Montreal, Quebec, Canada). The images were saved as bitmap files using Corel Draw™ (Corel Corporation, Ontario, Canada). The bitmap images were calibrated and the udder manually traced to identify the left and right halves of the udder. The image area in number of pixels, and the minimum, maximum and average temperatures, and the standard deviation of the average temperature were recorded and tabulated. Analysis of the data was performed using the computer programs Excel™ (Microsoft Corp., Redmond, Washington, USA) and SAS™ (SAS Institute Inc., Cary, North Carolina, USA).

The progression of mastitis development was objectively monitored using conventionally known tests such as the somatic cell count in the milk samples (Batra, T.R. and McAllister, A.J., 1984, J. Anim. Sci. 64: 305-312), BSA (Fernando, R.S. et al., 1985, J. Dairy Sci. 449-456), body temperature (Maatje, K. and Rossing, W., 1991, Mastitis Newsletter 16: 6-7), and presence of the enzyme N-acetyl-beta-D-glucosaminidase (NAGase) in the milk samples. NAGase is a lysosomal enzyme secreted in the mammary gland during inflammation. The presence of NAGase in milk is an indication of tissue damage (Perdigon, G. et al., 1986, J. Dairy Sci. 69: 27-31; Fang, W. et al., 1995, J. Dairy Sci. 79: 76-82; Losnedahl, K.J. et al., 1996, Illinois Dairy Report 1-4; Fang, W. and Pyorala, S., 1996, J. Dairy Sci. 79:76-82). By simultaneously testing standard indicators of mastitis and obtaining infrared thermographic images, it was possible to monitor the precise change in infrared characteristics parallel to the standard test results.

RESULTS

The results are presented in tabular form in Tables 1 and 2, and in graphical form in Figures 6-14. The treatments with experimental inflammation inhibitors were largely ineffective, and did not significantly change the mastitis response. Therefore, the data in Tables 1 and 2, and Figures 6-14, is not presented separately for each of the anti-inflammation treatment groups. Figures 6-9 provide least square means of data for the 20 animals tested. Figures 11-13 show separately the results obtained from one of the 20 animals tested, the individual animal (reference no. 5029) showing a false-negative result for mastitis when measured by rectal temperature rather than by infrared thermography. The same infrared thermographic ("IRT") data is depicted in each of Figures 6-9, plotted along with data obtained from various known techniques for detecting mastitis. Figure 14 provides the IRT data presented in the form of total temperature (mean temperature x image area or volume).

The results are most readily understood with reference to the figures. Figure 6 shows the mean temperature of the infrared thermographic image of the left distal quarter of the udder (induced) and the mean temperature of the infrared thermographic image of the right distal quarter of the udder (control) plotted over a 24 hour time course, together with rectal temperature plotted over the same time frame. Based upon the results depicted in Figure 6, the IRT data for the left and right distal quarters of the udder is very similar, although mastitis was induced only in the left distal quarter. One possible explanation for this is that the high heat transfer capacity through the water found in living cells accounts for the even temperature distribution observed between the distal quarters of the udder. The results from Figure 6 also indicate that the absolute change in temperature detected by IRT

is greater than that detected by measurement of rectal temperature, and that the rate of temperature change detected by IRT is greater than that detected by measurement of rectal temperature. The results in Table 1 indicate that the infrared thermographic image of the udder detected a statistically significant temperature difference ($p < 0.05$) by the 1 hour point after mastitis induction, whereas a significant difference in rectal temperature was not detected until much later (the 6 hour point after mastitis induction).

Figures 7, 8 and 9 plot the same IRT temperature information as in Figure 6, together with various standard measurements used in the detection of mastitis. Figure 7 shows the NAGase levels in the left and right distal udder quarters over the first 24 hours after induction of mastitis in the left distal quarter. As expected, the NAGase level in the left distal quarter increased sharply, indicative of mastitis, while there was little change in the NAGase level in the right distal quarter. As discussed earlier, given the separate vascular supplies of the quarters of the udder in cattle, an increase in NAGase level in the non-induced quarter would not be expected. Figures 8 and 9 depict similar results, showing, respectively, a significant increase in BSA level and somatic cell count in the left distal udder quarter and little or no change in the right distal quarter. Figures 7, 8 and 9 indicate that the mastitis induction model was indeed successful in inducing mastitis in the treated udder quarter, detectable by objective identifiers of mastitis, and that mastitis was also detected by IRT.

Figures 11, 12 and 13 emphasize the superior results that can be achieved by the methods of the invention over other temperature measurement techniques. These figures provide data for one of the test animals (animal no. 5029), in which rectal temperature remained nearly unchanged over the first 24 hours after induction of mastitis, whereas mean udder temperature as measured by IRT, changed significantly (Figure 11). Hence, in an animal in which measurement of rectal temperature disclosed a false-negative result, IRT of the udder correctly detected induced mastitis. Confirmation of induction of mastitis in animal no 5029 is documented in Figures 12 and 13 which show, respectively, significantly increased NAGase and BSA levels in the left distal quarter (induced) relative to the right distal quarter (non-induced).

Figure 10 shows the change in udder quarter area, as represented by number of pixels in an IRT image, for left (induced) and right (non-induced) distal udder quarters for 20 animals over the 24 hour period after mastitis induction. The data in Figure 10 is independent of temperature, and only refers to the number of pixels in a defined area of the image. It is apparent in Figure 10 that the swelling of the left distal quarter of the udder relative to the right distal quarter (resulting in a lack of symmetry) as a result of mastitis induction was readily detected from the IRT image.

Figure 14 combines IRT image area and mean image temperature as a total temperature (mean pixel temperature x number of pixels). In Figure 6, there was a very close symmetry between the IRT temperature of the left distal quarter and that of the right distal quarter, presumably due to the high heat transfer capacity of living cells. Conversely, in Figure 14, the left distal quarter (induced) exhibits a much higher total temperature than the right distal quarter (non-induced). The temperature information remains the same as in Figure 6, but the greater area of the portion of the image representative of the left distal quarter of the udder relative to the area of the right distal quarter (as a result of swelling in response to mastitis) is reflected in the total temperature measurement.

Referring again to Figure 6 and to Table 1, it will be appreciated that the mean IRT image temperature at the time - 1 h (1 hour before induction of mastitis) reflects the IRT image temperature of the udder when the animals do not have mastitis, and therefore acts as a control IRT temperature for the animals in a healthy state. In the period from 3 hours post-induction and 12 hours post-induction, the mean IRT temperature for both the left and right hind udder quarters for the 20 animals was less than 1 °C greater than the control value of 32.19°C. Hence, an IRT udder temperature less than 1 °C greater than a control value for an animal in a healthy state is indicative of mastitis in a subject mammal.

Figure 6 and Table 1 shows that, during the first 24 hours after induction of the mastitis model, mean IRT temperature for both the left and right distal udder quarters for the 20 animals tested changed at a rate of at least 0.1 °C per hour, whether increasing or decreasing. Hence, a rate of change of IRT temperature of at least 0.1 °C per hour is indicative of mastitis in a subject mammal.

Figure 10 shows that during the first 24 hours after induction of mastitis in the left distal quarter of the udder, the area of the portion of the image corresponding to the induced quarter is at least 10% greater than that of the non-induced (control) right distal quarter of the udder. Thus, if the area of a portion of the image corresponding to a first quarter of the udder of the animal differs from the area of a portion of the image corresponding to a second quarter of the udder of the animal by greater than 10%, this is indicative of mastitis in the animal.

Similarly, referring to Figure 14 and Table 2, during the first 24 hours after induction of mastitis in the left distal quarter of the udder, the total temperature (mean pixel temperature x number of pixels) of the portion of the image corresponding to the induced quarter is at least 10% greater than that of the non-induced (control) right distal quarter of the udder. Thus, if the total temperature of a portion of the image corresponding to a first quarter of the udder of the animal differs from the total temperature of a portion of the

image corresponding to a second quarter of the udder of the animal by greater than 10%, this is indicative of mastitis in the animal.

Table 1: Time course for infrared temperature measured by infrared thermography, rectal temperature and milk analysis parameters in cows utilized in a mastitis induction model (n=20). Data represent least squares means.

Time (h)	Rectal Temp °F	Infrared Temp °C	NAGase μg/ml	Somatic Cell Counts	BSA g/dl
-1	101.2a	32.19a	0.39a	504a	0.329a
0.5	101.3a	32.36ab			
1	102.0a	32.77bc			
2	102.0a	32.97cd			
3	102.7a	33.76e	2.39b		2.86b
6	105.3b	34.44f	5.66b		4.17b
9	102.2a	34.94d	5.15b		3.13b
12	96.7b	33.42d	4.58b		2.35b
24	100.9a	30.99	5.64b	2875b	2.76b
36	101.2	33.15	5.59b	2753b	1.50b
48	101.1	31.43	4.72b	1849b	0.87a
60	101.7	33.11	3.46b	1370b	1.03a
72	101.1	31.68	2.44a	933a	0.67a

a,b, – means with different letters within columns are significantly different ($P<0.05$)

Table 2: Time course for mean total temperature values (infrared thermographic temperatures X udder area in pixels) for left, distal udder quarter (mastitis induced) and right, distal udder quarter (non-induced) in lactating dairy cows. Values represent least squares means for 20 cows.

Time	Total Temperature Values for left (induced) and Right (non-induced; control) Udders		
(h)	Left	Right	
-1	52755 a	51486 a	X
3	77553 b P=0.001	62395 b P=0.002	Y
6	81294 b P=0.001	63998 b P=0.001	Y
9	79250 b P=0.001	66237 b P=0.001	Y
12	66017 b P=0.002	53782 a P=0.50	Y
24	56916 a P=0.23	50630 a P=0.81	Y
36	60989 b P=0.02	54157 a P=0.44	Y
48	59322 b P=0.06	54015 a P=0.47	X
60	61971 b P=0.008	55370 a P=0.26	X
72	56745 a P=0.25	55571 a P=0.24	X

a,b, – means with different letters within columns are significantly different ($P<0.05$)

X,Y, – means with different letters within rows are significantly different ($P<0.05$). Left is the mastitis induced distal quarter, right is the distal, non-induced quarter (control).

The present invention is not to be limited in scope by the specific embodiments described herein, which are intended as single illustrations of individual aspects of the invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

All references cited herein are incorporated herein by reference in the entirety for all purposes.